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Application of fluorescent protein ATeam for ATP level measurements in living bacteria

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ATeam is a fluorescent ATP indicator based on FRET and composed of the ϵ subunit of bacterial F_0F_1 -ATP-synthase sandwiched by the cyan and yellow fluorescent proteins [1]. It turned to be convenient for visualizing of ATP levels inside living eukaryotic cells, but to our knowledge it was never used in experiments on bacteria. The intracellular ATP level is a key parameter in bacterial bioenergetics, so we consider ATeam as an efficient tool in this field.

Using ATeam probe, we developed a new method for ATP level measurement in bacterial living cells. We monitored the probe response in wild type *Escherichia coli* cells expressing ATeam, and tested its adequacy with standard chemiluminescence method. In this system, as expected, the signal rate increased after glucose addition and was reduced after addition of ATP synthesis inhibitor potassium arsenate. The addition of respiratory substrate succinate also led to signal rate increase, and the following addition of protonophore resulted in decrease of signal rate. When uncoupling preceded the succinate addition, the signal rate increase was not observed.

With new approach, we also studied dynamics of ATP levels in *Escherichia coli* Δunc mutant with deleted ATP synthase operon. Glucose and arsenate additions influenced the signal rate the same way as in wild type. Addition of succinate to mutant cells unexpectedly led to increase of signal rate, which, however, was half less than that after glucose addition.

In general, our data correspond well to general knowledge of bacterial energetic metabolism, so our method seems suitable for bacterial bioenergetic studies.

Reference

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Predicted topology of the ion-conducting subunit-a of the membrane motor of the ATP synthase

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Subunit-a plays a critical role in the rotary mechanism of the ATP synthase F_0 membrane motors. First, it functions as the stator against which the c-subunit ring rotates, thus producing the mechanical torque required for catalysis in the soluble F_1 motor. Second, it provides access pathways for protons or Na^+ ions to and from the c-ring binding sites, thereby coupling the rotation of the c-ring to the transmembrane electrochemical gradient. The structure of subunit-a

is to date unknown, but it is generally accepted that a bundle of four transmembrane helices, TM2-TM5, lie in proximity to the c-ring. However, the topology of this bundle, which likely influences the ion-translocation mechanism, remains unclear. Here, we first use the structure-prediction algorithm Rosetta to construct a topological model of the *E. coli* aTM2-aTM5 helix bundle that is optimally consistent with existing cysteine cross-linking data for the isolated subunit-a. We also construct a model of the a/c-interface, guided by novel cross-linking data between genetically fused c2-subunits and subunit-a, plus previously reported cross-linking and accessibility data involving aTM4-aTM5 and the c-subunit. Our results demonstrate that the aTM2-aTM5 transmembrane bundle is organized in a clockwise manner viewed from the cytoplasm. In our a/c-complex model we identify two half-channels from the center of the membrane, one at the a/c-interface extending towards the cytoplasm and another within the subunit-a bundle, open to the periplasmic environment.

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ATP synthase from rat liver plasma membrane

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Over the past few years, a growing number of reports, including our work [1], have described the presence of F_0F_1 ATP synthase subunits at the surface of a wide variety of cells, including hepatocytes. Expression of ATP synthase at the cell surface is related to different biological effects; in liver it mediates HDL endocytosis through the activation of the G protein-coupled purinergic receptor P2Y13 [2].

A question regarding the ectopic ATP synthase is its sub-localization at the plasma membrane. Hepatocytes are polarized due to the presence of the basolateral and canalicular membranes, devoted for uptake from the portal blood and bile secretion, respectively. Here we have compared two preparations of plasma membrane [1, 3] from rat liver, which have been characterized for their content of basolateral membrane markers. Both preparations were fractionated using different concentrations of digitonin, followed by hr CN PAGE, and the presence of F_0F_1 complexes was established by in-gel activity staining and immunoblotting.

The results indicate that the plasma membrane preparation enriched in basolateral proteins contains higher amounts of complete and active F_0F_1 complexes, in accordance with their specific function to modulate HDL uptake on hepatocyte surface. Moreover, we have demonstrated that, independently of the extraction protocol, ATP synthase complexes display a similar molecular weight to the monomeric form of the mitochondrial ATP synthase. This confirms that, in the plasma membrane, the F_0F_1 complexes do not associate into dimers and oligomers as they do in mitochondria.

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References

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